Göteborg, 2019

Understanding the role of long non-coding RNAs in neuroblastoma development and progression

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet, kommer att offentligen försvaras i hörsal **Arvid Carlsson**, Academicum, Medicinaregatan 3, Göteborg

Tisdagen den **3 December** 2019, klockan **9.00**

Av **Sanhita Mitra**

Fakultetsopponent: Dr. Valérie Castellani
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Avhandlingen baseras på följande delarbeten

I. Pandey GK*, **Mitra S***, Subhash S, Hertwig F, Kanduri M, Mishra K, Fransson S, Ganeshram A, Mondal T, Bandaru S, Ostensson M, Akyürek LM, Abrahamsson J, Pfeifer S, Larsson E, Shi L, Peng Z, Fischer M, Martinsson T, Hedborg F, Kogner P, Kanduri C. (2014). The risk-associated long noncoding RNA *NBAT1* controls neuroblastoma progression by regulating cell proliferation and neuronal differentiation. *Cancer Cell*, *26*(5), 722-737. doi:10.1016/j.ccell.2014.09.014 (* Co-first author)

II. Mondal T, Juvvuna PK, Kirkeby A, **Mitra S**, Kosalai ST, Traxler L, Hertwig F, Wernig-Zorc S, Miranda C, Deland L, Volland R, Bartenhagen C, Bartsch D, Bandaru S, Engesser A, Subhash S, Martinsson T, Carén H, Akyürek LM, Kurian L, Kanduri M, Huarte M, Kogner P, Fischer M, Kanduri C. (2018). Sense-Antisense lncRNA Pair Encoded by Locus 6p22.3 Determines Neuroblastoma Susceptibility via the USP36-CHD7-SOX9 Regulatory Axis. *Cancer Cell*, *33*(3), 417-434 e417. doi:10.1016/j.ccell.2018.01.020

III. **Mitra S**, Muralidharan SV, Di Marco M, Juvvuna PK, Kosalai ST, Raimondi I, Huarte M, Kogner P, Fischer M, Johnsen JI, Mondal T, Kanduri C. A p53 responsive lncRNA *NBAT1* determines chemotherapeutic response in neuroblastoma through regulating p53 sub-cellular distribution (Manuscript)

SAHLGRENSKA AKADEMIN
INSTITUTIONEN FÖR BIOMEDICIN

GÖTEBORGS UNIVERSITET

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Understanding the role of long non-coding RNAs in neuroblastoma development and progression

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Abstract

Neuroblastoma (NB), a common cancer of childhood, contributes to 15% of all pediatric cancer deaths. The improper neuronal differentiation of neural crest cells to mature neurons in the sympathetic nervous system leads to NB tumor formation. NB is an extremely heterogeneous disease and high-risk NB is very difficult to treat, with the incidence of relapse in 50% of cases despite of intensive chemotherapeutic treatment. Long non-coding RNAs (lncRNAs) are a class of biological molecules that are transcribed but not translated to any functional protein. The mechanism of functions for these lncRNAs are diverse and context-specific. De-regulation of lncRNAs has been proposed to play a critical role in cancer development and progression. The goal of the current thesis was to identify novel neuroblastoma-specific lncRNAs for better stratification of the disease and characterizing their functional role in greater detail.

In the first study, we characterized differentially expressed lncRNAs between low-risk and high-risk NB tumors using transcriptome profiling. Among the differentially expressed lncRNAs, we chose a lncRNA, neuroblastoma associated transcript 1 (*NBAT1*), that maps to NB hotspot locus, 6p22.3, which has been shown to harbor several NB-specific risk-associated SNPs. We showed that *NBAT1* is a tumor suppressor lncRNA and it carries out this tumor suppressor function through regulating cellular proliferation and differentiation. Consistent with its tumor suppressor properties, its higher expression in NB patients predicts a good prognosis. Mechanistically, *NBAT1* controls NB cell growth through epigenetically silencing cell proliferating genes, as well as NB cell differentiation by repressing the neuron-restrictive silencer factor NRSF, also known as REST.

In the second study, we sought to investigate the functional connection between *NBAT1* and its sense partner *CASC15* lncRNA in NB development and progression. Like *NBAT1*, *CASC15* harbors NB-specific tumor suppressor properties and its higher expression in NB patients correlates with good clinical outcomes. We show that *CASC15*/*NBAT1* (6p22lncRNAs) promote cell differentiation by the specific regulatory interactions with *SOX9* and USP36 located on 17q, which is frequently gained in NB. We could show mechanistically that 6p22lncRNAs dictate *SOX9* expression by controlling CHD7 stability via modulating cellular localization of USP36, which is a deubiquitinase.

In the third and final study, we found that *NBAT1* is a p53 responsive lncRNA and regulates p53 subcellular localization. We observed that a decrease in *NBAT1* expression in NB cells results in resistance to genotoxic drugs, which in part occurs due to cytoplasmic p53 accumulation and concomitant loss of p53 dependent gene expression. Higher expression of the p53 exporter CRM1 in *NBAT1* depleted cells contributes to p53 cytoplasmic localization, while CRM1 inhibition in these cells restores p53 localization. We observed that combined inhibition of CRM1 and *MDM2* sensitized aggressive NB cells with cytoplasmic p53, suggesting that this drug combination could be a potential therapeutic strategy for high-risk NB patients.

In summary, these findings highlight the regulatory role of lncRNAs in NB disease development.

Keywords: Neuroblastoma, Long non-coding RNAs, *NBAT1*, NRSF/REST, CASC15, chromosome 6p22, *SOX9*, USP36, CHD7, p53, CRM1, MDM2.

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